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Bone Marrow Treatment of Lethally Irradiated with Gamma-Rays under High Dose Rate (VII)

Effects of Preceding Thymectomy on Syngeneic and Allogeneic Bone Marrow Transplantation

Hiroshi TAZAWA, Morihisa YAMAGISHI and Gyoichi WAKISAKA*

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1) Experimental schedule attempting to prevent secondary disease by preceding thymectomy was presented.

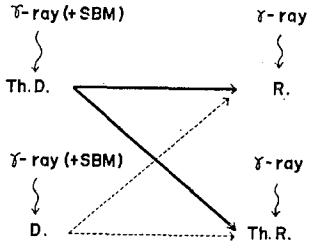
2) It was confirmed that the thymus played an essential role for establishing adoptive immunity in syngeneic radiation chimeras.

3) No effect of preceding thymectomy on survival rates was observed in syngeneic chimeras.

4) The procedure appeared to be useful for the suppression of the secondary disease in allogeneic chimeras, bebause the survival rate of thymectomized recipients was better than non-thymectomized one at 45 days after irradiation, and the difference was statistically significant.

INTRODUCTION

It has already been shown that the thymus is essential not only for proper development of immunological competence in neonatal mice, but also for complete recovery from radiation damage of immunological potency in adult mice. In our laboratory, experiments have been planned to estimate the role of the thymus



D : Bone marrow donor
Th.D : Thymectomized bone marrow donor
R : Bone marrow recipient
Th.R : Thymectomized bone marrow recipient
SBM: Syngeneic bone marrow

Fig. 1. Scheme of experiments.

* 田沢　熙，山岸　司久，脇坂　行一：The First Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto.

played on adoptive immunity in syngeneic radiation chimeras, and on the incidence and pathology of secondary disease in allogeneic ones.

The experimental schedule is illustrated in Fig. 1. If thymectomized mice fail to recover immunological competence from radiation damage, the following possibilities are considered. When these immunologically incompetent mice are used as donors for allogeneic bone marrow transplantation, graft-versus-host reaction (GVHR) may be protected. On the other hand, if they are used as recipients, bone marrow graft may take easier, and the acquisition of immunological competence by the precursor cells in graft will not take place, thus again GVHR may be prevented. In the present report some results obtained so far are to be described.

MATERIALS AND METHODS

Animals were female Dd/s, C3H and Na2 inbred strain mice supplied from Kyoto University Inbred Animal Center. At the age of 6 to 8 weeks, they were thymectomized by means of superior sternotomy and suction. The procedure was checked histologically at post-mortem examination. Irradiation was performed by the Co^{60} gamma-irradiation facility belonging to the Institute for Chemical Research of Kyoto University. Radiation doses were sublethal (500 R. or 700 R.) in some control experiments, but mostly were lethal. Lethal doses means 1000 R. for Dd/s strain mice, and 900 R. for Na2 strain. The facility could deliver 0.6602×10^5 R/hr. of radiation in January, 1968. The other conditions of irradiation were described elsewhere[1]. Within 4 hours after irradiation, syngeneic or allogeneic bone narrow cell suspension in Tyrode's solution was infused intravenously. The combination of donor's and recipient's strain, and the cell number infused are shown in Tables 1 and 2.

Immunological responsiveness were tested by the titration of anti-sheep red blood cell hemagglutinin and by the survival time of skin allograft. The sheep blood in Alsver's solution was purchased from Nakarai Chemicals Ltd., Kyoto. As donors of skin allograft, C3H strain mice were used. Experimental animals were weighed every two days. The survival rate was compared between the thymectomeized chimeras and the non-thymectomized ones.

RESULTS

1. Syngeneic Chimeras

The survival rates of the thymectomized and the non-thymectomized mice which were lethally irradiated and treated with syngeneic bone marrow cells are summarized in Table 1. The survival rate of thymectomized recipients at 30 days after irradiation was 82.0 per cent. Difference of 30 day survial between the thymectomized group and non-thymectomized one appeared to be negligible, and harmful effect of thymectomy on survival rate and body weight changes was hardly observed.

Recovery of immunological potency from radiation damage was tested with two methods as described above. The production of anti-sheep RBC hemagglu-

Table 1. Survival rate of syngeneic chimeras.

Recipient	Donor	No. of B. M. cells infused $\times 10^6$	No. of living / No. of irradiated (%)						
			days after irradiation						
			7	14	21	30	60	90	120
Dd/s	Dd/s	5 to 7	25/25 (100)	24/25 (96.0)	24/25 (96.0)	24/25 (96.0)	12/12 (100)	4/4 (100)	*
Thx.Dd/s	Dd/s	5 to 7	48/52 (92.3)	44/51 (86.3)	42/50 (84.0)	41/50 (82.0)	18/29 (62.1)	14/23 (60.9)	8/21 (38.1)*
Dd/s	—	—	33/36 (91.7)	8/36 (22.2)	5/36 (13.9)	1/36 (2.8)	0/36 (0)		

B. M.: Bone marrow. Thx. Dd/s: Thymectomized Dd/s.

* Combined data of several groups. Some of them were excluded from the survival study after 30 th day because of using them for other experiments.

tinin at 7 days after primary sensitization was shown in Fig. 2. Although the non-thymectomized mice at 4 weeks after lethal irradiation and syngeneic bone marrow transplantation reacted to sheep RBC less than non-irradiated mice, their reactivity recovered fully at 7 weeks. The previously thymectomized mice, on the other hand, reacted significantly less than non-thymectomized controls at 4 weeks, and the weak reactivity continued for as long as 4 months. This difference was observed in the experiments with 700 R. irradiation, but not with 500 R. As shown in Fig. 3, rejection of skin allograft in the thymectomized mice was obviously delayed only in the experiment with 1000 R. irradiation.

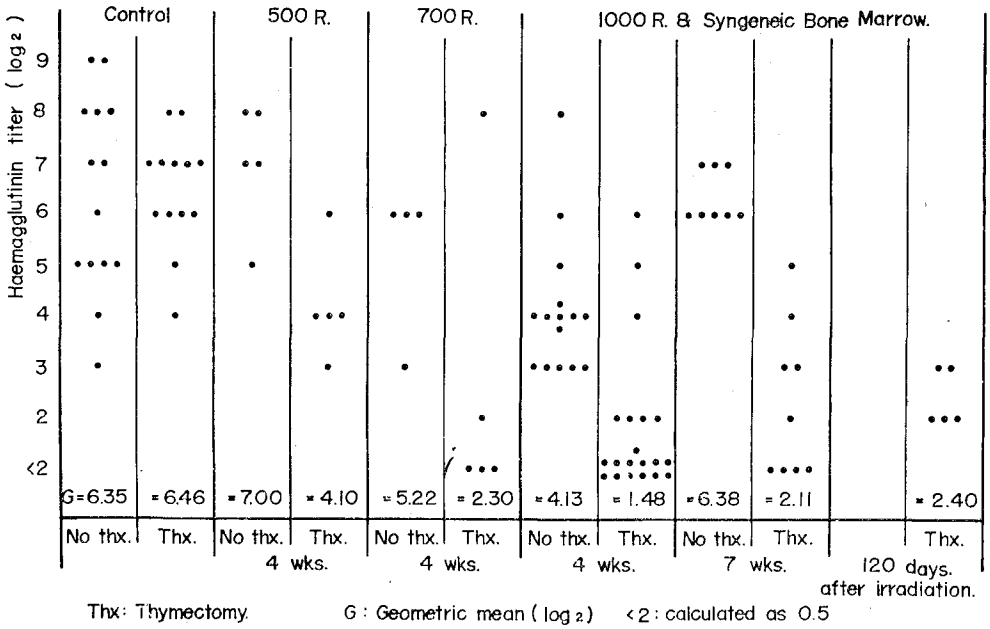


Fig. 2. Immune response of thymectomized and non-thymectomized mice to sheep RBC after gamma-irradiation (and syngeneic bone marrow treatment).
—*Primary response*—

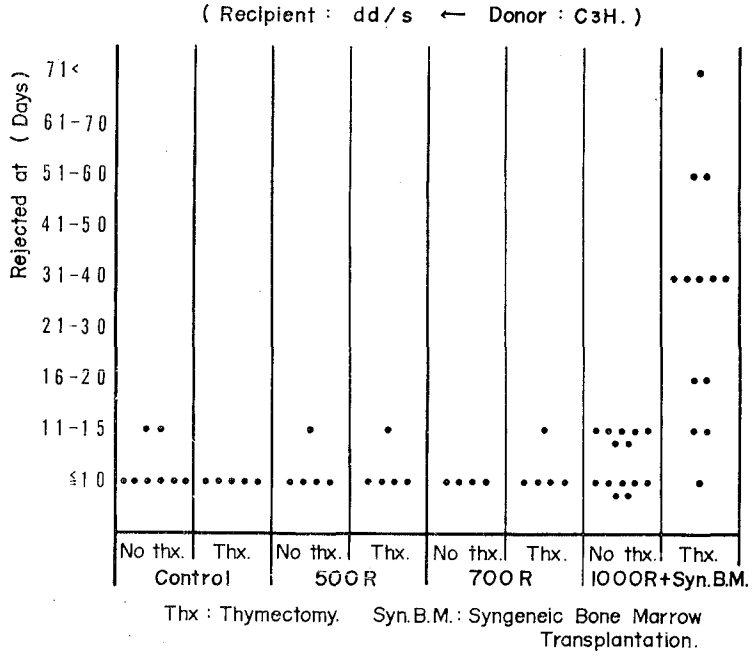


Fig. 3. Immune response of thymectomized and non-thymectomized mice to skin allograft 4 weeks after gamma-irradiation.

2. Allogeneic Chimeras

Na2 and Dd/s strain mice were used as donors and recipients respectively. Abundant informations concerning with this combination have been obtained during last 9 years in our laboratory[2~9]. The survival rates are summarized in Table 2. The survival of the mice treated with 5 to 7×10^6 bone marrow cells was poor; besides, the thymectomized recipients died earlier. The mice treated

Table 2. Survival rate of allogeneic chimeras.

Recipient	Donor	No. of B. M. cells infused $\times 10^6$	No. of living / No. of irradiated (%)					
			days after irradiation					
			7	14	21	30	45	60
Dd/s	Na2	5 to 7	20/21 (95.3)	10/21 (47.6)	10/21 (47.6)	6/21 (28.6)	2/21 (9.5)	2/21 (9.5)
Thx.Dd/s	Na2	5 to 7	15/18 (83.4)	7/18 (38.9)	4/18 (22.2)	3/18 (16.7)	2/18 (11.1)	1/18 (5.6)
Dd/s	Na2	18 to 20	37/39 (94.8)	35/37 (94.6)	31/36 (86.2)	18/35 (51.4)	6/34 (20.2)	3/34 (8.8)
Thx.Dd/s	Na2	18 to 20	37/38 (97.4)	31/36 (86.2)	24/35 (68.6)	20/34 (58.8)	15/33 (45.5)	5/33 (15.2)
Dd/s	Thx.Na2 900R. SBM	5 to 7	26/26 (100)	17/26 (65.4)	8/26 (30.8)	5/26 (19.4)	2/26 (7.7)	1/26 (3.8)
Thx.Dd/s	Thx.Na2 900R. SBM	5 to 7	17/18 (94.5)	12/18 (66.7)	8/18 (44.4)	6/18 (33.3)	6/18 (33.3)	4/18 (22.2)

Thx. Na2 900 R. SBM. : Thymectomized, 900 R. irradiated and syngeneic bone marrow transplanted Na2.

with 18 to 20×10^6 bone marrow cells survived better. Some differences of survival rate were observed between the thymectomized recipients and the non-thymectomized ones in the experiment of transplanting 18 to 20×10^6 cells. The survival rate of the thymectomized recipients was lower during the first 4 weeks after irradiation, but became better afterwards than that of the non-thymectomized ones. Although the difference at 21 days is statistically insignificant ($P=0.13$), that of 45 days is significant ($P=0.02$), suggesting GVHR may be suppressed by the preceding thymectomy.

5 to 7×10^6 bone marrow cells obtained from thymectomized, lethally irradiated and syngeneic bone marrow cell transplanted Na2 mice did not save satisfactorily either thymectomized or non-thymectomized recipients.

DISCUSSION

The fact that the thymus plays an important role for adoptive immunity in syngeneic radiation chimeras was confirmed in the experiment, as Miller *et al.*[10~13] and many investigators have already shown.

The immunologically suppressed chimeras were apprehended to die readily from invasions of micro-organisms. In spite of these expectation their survival rates were fairly good, and so-called wasting which occurred usually in neonatally thymectomized mice was never observed in the present experiment. Perhaps, these mice are restrictively immunocompetent from two possible reasons. The first, the bone marrow is considered to contain a small number of thymus independent precursors of the immunologically competent cell. Because these thymus independent precursors may contribute to reconstituting immunological potency to some degree in the thymectomized chimeras, they have ability to resist to frequent attacks of infections. The second, a small number of imunocompetent cells in host's lymphoid tissues have a narrow escape from radiation damage and may restore immunological potency of the mice.

Many reports[14~19] concerning the effect of preceding thymectomy on allogeneic or xenogenic hematopoietic cell transplantation have been presented, but their results varied considerably. Van Putten[14] reported a beneficial effect protecting the secondary disease in xenogeneic bone marrow transplantation. Simmons *et al.*[15] could not find any effect on the survival rate of allogeneic radiation chimeras. Good and others of Minnesota group[17~19] demonstrated the promoting effect of neonatal thymectomy on the susceptibility to GVHR in the experiments of intraperitoneal spleen cell injection.

In the present experiment, the effect of thymectomy on the survival rates of the allogeneic radiation chimeras was biphasic, if to say daringly. During the first 4 weeks after irradiation, the survival rate of thymectomized recipients was less than that of non-thymectomized ones, but it became better afterwards. Although the former difference is statistically insignificant, the latter is significant. This significant difference seems to be caused by the suppression of GVHR in the thymectomized chimeras, since the precursor cells are thought to fail to acquire immunological competence in thymectomized host.

It is speculated that there are two types of precursors of immunologically competent cells in the bone marrow, namely thymus dependent (PCo) and thymus independent (PC_I), and PCo greatly outnumbers PC_I in the organ[20]. Then the thymus is considered to be necessary for the transplanted bone marrow to confer adoptive immunity. However, whether or not the thymus plays the same important role in allogeneic bone marrow grafting as in syngeneic one has not been settled yet[15,21].

In the bone marrow of thymectomized syngeneic radiation chimeras, the ratio of PC_I to PCo is supposed to be lees than normal. Then, as shown in Fig. 1, if these treated mice are selected as candidates of allogeneic bone marrow donors, the suppression of GVHR may be more effective. Experiments are now being carried out to test these possibilities.

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